IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants:	Joseph P. Vacanti, Christopher K. Breuer, Beverly E. Chaignaud, and Toshiraru Shin'oka		
Serial No.:	10/782,750	Art Unit:	3738
Filed:	February 19, 2004	Examiner:	David J. Isabella
For:	*ENGINEERING OF STRONG, PLIABLE TISSUES*		

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 1-5, and 8-15 in the Office Action mailed June 8, 2006, in the above-identified patent application. A Notice of Appeal was filed on September 8, 2006.

The Commissioner is hereby authorized to charge $310.00, the sum fee for the filing of this Appeal Brief and a Petition for an extension of time for one month, up to an including December 8, 2006, for a small entity, to Deposit Account No. 50-3129. It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

(1) REAL PARTY IN INTEREST

The real party in interest of this application are the assignee, Children's Medical Center Corporation, MA and the licensee, Tengion Inc., PA.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to the appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1-5 and 8-17 are pending and on appeal. Claims 6 and 7 have been cancelled. Claims 16 and 17 have been withdrawn and re-filed in a divisional application. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

The claims were last amended in the amendment filed on October 3, 2005. Claims 6 and 7 were cancelled and new claims 8-15 added in the Preliminary Amendment filed on February. 2, 2004. Claims 16 and 17 were added in the Amendment and Response filed on October 3, 2005 and withdrawn in the office action mailed on December 13, 2005.

(5) SUMMARY OF THE CLAIMED SUBJECT MATTER

Independent claim 1 defines a method for making a cell-matrix construct for use as a heart valve comprising

implanting into an animal a cell-matrix construct comprising a fibrous matrix (column 3, lines 42-51) in the shape of a heart valve or heart valve leaflet (column 7, lines 21-23, example 1, columns 7-8, example 6, column 11), wherein the matrix is formed of a biocompatible, biodegradable polymer (column 4, lines 2-7) having seeded therein cells selected from the group consisting of endothelial cells, myofibroblasts, skeletal muscle cells, vascular smooth muscle

cells, myocytes, fibromyoblasts, and ectodermal cells (column 6, lines 26-34), wherein the cell-matrix construct can withstand repeated stress and strain (column 2, lines 40-42, column 5, lines 31-43, column 7, lines 12-29). The matrix can also be seeded with dissociated parenchymal or connective tissue cells (claim 2, original claim 5, column 6, lines 26-34). The matrix can first be cultured at a first site in a patient prior to being transplanted to a second site (claim 3, column 3, lines 3-9).

Claim 8 requires the heart valve have mechanical strength, and flexibility or pliability (column 2, lines 48-55, column 3, lines 62-67, column 7, lines 12-29).

The cell-matrix construct is formed of a polymer selected from the group consisting of poly(lactide) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, and degradable polyurethanes (claim 9) or of a polymer selected from the group consisting of polyacrylates, ethylene-vinyl acetate polymers, acyl substituted cellulose acetates, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, and nylon (claim 10, column 4, line 1, until column 5, line7).

As recited in claim 11, the cell-matrix construct contains interconnected pores in the range of between approximately 100 and 300 microns (column 3, lines 45-46), it can include growth factors (claim 12), such as those listed in claim 13 (column 5, lines 59-67). The cell-matrix can further comprise bioactive factors incorporated to between one and 30% by weight (claim 14; column 5, lines 45-49 and column 6, lines 4-13).

Claim 15 defines the cell-matrix construct of any one of claims 1-14.

Claim 16 defines a cell-matrix construct for use as a heart valve or heart valve leaflet comprising

a fibrous polymeric matrix in the shape of a heart valve or heart valve leaflet, wherein the matrix is formed of a biocompatible, biodegradable polymer having seeded thereon cells comprising myofibroblasts grown to confluence and then endothelial cells seeded thereon, which as required by claim 17, can withstand repeated stress and strain (column 7, line 53, until column 8, line 9, column 2, lines 37-45).

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issue presented on appeal is:

(a) whether claims 1-5 and 8-15 are obvious under 35 U.S.C. §103(a) over U.S. Patent No. 3,514,791 to Sparks, et al. ("Sparks") in view of U.S. Patent No. 5,514,378 to Mikos ("Mikos") or U.S. Patent No. 5,709,854 to Griffith-Cima, et al. ("Griffith") and in view of either U.S. Patent No. 4,795,459 to Jauregui ("Jauregui") or U.S. Patent No. 4,916,193 to Tang, et al. ("Tang").

(7) ARGUMENTS

(a) The Claimed Invention

Despite advances in its treatment over the past years, valvular heart disease is still a major cause of morbidity and mortality in the United States, killing thousands of Americans each year. Heart valve replacement with either nonliving xenografts or mechanical prostheses is an effective therapy for valvular heart disease. However, both types of heart valve replacements have limitations, including finite durability, foreign body reaction or rejection and the inability of the non-living structures to grow, repair and remodel.

Many tissues have been engineered using methods in the prior art, which utilize porous structures which support cell growth throughout a biocompatible and biodegradable matrices. However, there remains a need to improve the characteristic mechanical and physical properties

of resulting tissues obtained from the resulting tissue, which in some cases do not possess the requisite strength and pliability to perform its necessary function *in vivo*.

Appellants have discovered a method of making cell-construct using biocompatible, biodegradable polymer matrices, which can withstand repeated stress and strain for use as heart valves or heart valve leaflets. The method is an improvement over the prior art, using the recipient or an animal as the initial bioreactor to a form fibrous tissue-polymeric constructs which can be seeded with other cells and implanted.

(b) Rejections Under 35 U.S.C. § 103(a)

The Legal Standard

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177 (C.C.P.A. 1967); *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992); *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989).

This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

It is clear that to establish a rejection under 35 U.S.C. §103 the cited references must (1) recite each element of the claims, (2) provide one of skill in the art with the motivation to modify the cited reference and (3) provide one of ordinary skill in the art with a reasonable expectation of success.

The Prior Art

Sparks

Sparks describes a die into which is placed a Dacron mesh secured to a stainless steel supporting ring (see column 5, lines 18-24). The die consists of a tube and mandrel (col. 3, lines 29-31). The die may be seeded with cells to make special parts, periosteal cells being used to make bone and epithelial cells being used to make epithelial tissue (abstract). Figures 6-12 illustrate a die for growing a tricuspid heart valve. Sparks further discloses that natural body processes produce the necessary connective tissue to fill the die cavity and form the valve graft (see column 2, lines 27-32 and column 5, lines 32-36).

Mikos

Mikos discloses biocompatible porous polymer membranes prepared by dispersing salt particles in a biocompatible polymer solution. Mikos discloses that a three dimensional structure can be manufactured from the membranes. The resulting three-dimensional foam or shape is a porous, biocompatible matrix to which cultured cells can attach and proliferate, and can be used for organ transplant or reconstructive surgery (Mikos, column 3, lines 25-45).

Griffith

Griffith discloses a cell-polymeric solution which is injected into an animal where the polymer crosslinks to form a polymeric hydrogel containing dispersed cells and the cells form new tissue in the animal. The hydrogel solution containing the cells can be injected directly into a patient where it hardens into a matrix having cells dispersed therein, or the hydrogel is poured into a mold having a desired anatomical shape, then hardened, which can be implanted into a patient (Griffith, column 1, lines 42-61).

Jauregui

Jauregui discloses an implantable prosthetic device made of biocompatible polymer and having a substantially continuous layer of autologous living cells attached via oligosaccharide-lectin recognition linkages (abstract).

Tang

Tang discloses totally or partially bioabsorbable devices capable of degrading into biologically innocuous components.

Analysis

Claims 1-5 and 8-15 are not obvious over Sparks in view of Mikos or Griffith and in view of Jauregui or Tang.

Claims 1, 2, 4 and 5

The claims define a method of making a cell-matrix construct for use as a heart valve or heart leafletcomprising implanting into an animal a cell-matrix construct comprising

a fibrous matrix in the shape of a heart valve or heart valve leaflet,

wherein the matrix is formed of a biocompatible, biodegradable polymer

having seeded therein cells selected from the group consisting of endothelial cell, myfibroblasts, skeletal, vascular smooth muscle cells, myocytes, fibromyoblasts and ectodermal cells,

wherein the cell-matrix construct can withstand repeated stress and strain (claim 1). Claim 2 recites the added limitation that the matrix is seeded with dissociated parenchymal or connective tissue cells.

The references cited by the Examiner do not recite all the claim limitations. Sparks does not disclose a method for making cell-matrix constructs for use as a heart valve as defined by claim 1. Sparks does not disclose or suggest using a fibrous polymeric matrix in the shape of a heart valve or heart valve leaflet which is implantable. Sparks does not disclose how to make a cell-matrix construct which can withstand repeated stress and strain. None of the secondary references make up for these deficiencies.

Sparks discloses a stainless steel die for growing a heart valve. A die cavity is formed between the outer and inner die members, and it is in this die cavity that the heart valve is grown (see Sparks, column 5, lines 18-20). A mesh reinforcing member is placed in the cavity, and connective tissue entering the die cavity through perforations in the outer die member encapsulates the mesh and completely fills the die cavity to form a heart valve which is similar in shape to the mesh. Thus the graft has a rigid circular base rim containing a steel ring (Sparks, column 5, lines 32-40). It is clear from the disclosure in Sparks, that the method of forming the graft involves implanting a stainless steel die in an animal, containing Dacron mesh for reinforcement. Therefore, a *prima facie* case of obviousness has not been established, since the references (when combined) do not teach or suggest all the claim limitations.

The Examiner contends that claim 1 does not preclude the use of additional structure including supporting or framework for supporting the fibrous matrix in keeping with the claimed

method and the construct of Sparks, and that one of ordinary skill in the art would equate the fibrous matrix of the claim to be equivalent in structure and function to the Dacron mesh of Sparks. Although a claim must be given its broadest possible meaning, this interpretation must also be consistent with the specification (MPEP §2111). Claim 1 recites a fibrous matrix ***formed of a biodegradable polymer*** which is capable of assuming a three dimensional structure of a heart valve of heart valve leaflet. Sparks discloses a non-biodegradable mesh and stainless steel die. One skilled in the art would be led by this disclosure to not use a biodegradable material. In contrast, appellants' specification discloses multiple examples demonstrating that the polymers themselves are molded into the three dimensional structures needed for the particular structure to be engineered and that they must degrade completely. Indeed, an important feature is that the polymers must be in the shape of the implant to be made. See, for example, column 3, lines 10-24. The Dacron mesh employed in Sparks is used for reinforcement (please see Sparks, see column 2, lines 9-10). A skilled artisan would recognize that the Dacron mesh used in Sparks is not analogous in structure or function (it is used for reinforcement as opposed to a biodegradable scaffold shaping the implant while the cells grow, then disappearing; please see column 3, lines 25-41 of the specification) with the fibrous matrix recited in the claims.

Sparks discloses that when a graft of epithelial tissue is desired, the die may be seeded with epithelial cells (column 4, lines 3-10). Sparks also states that before implanting a bone graft die, it is seeded with periosteal cells (column 4, lines 50-60). The Examiner has interpreted these disclosures to mean that the cells are seeded in the Dacron mesh and this is therefore the same as seeding cells in a fibrous matrix. This is not accurate, however. Even if this were the case, Sparks does not disclose seeding the graft for a heart valve with cells. Sparks does not teach that the fibrous matrix must be in the shape of a heart valve or heart leaflet. It is not clear how this can be accomplished using a Dacron mesh fitted in the space between the die members 40 and

42, shown in figure 7 of Sparks as 51. Also, as noted above, Dacron mesh is not biodegradable, a characteristic required by claim 1. The Board's attention is respectfully drawn to the claim language, which recites "a method of making a cell-matrix construct for use as a heart valve, comprising implanting into an animal a cell-matrix construct comprising a fibrous matrix in the shape of a heart valve or heart valve leaflet".

Contrary to the Examiners' assertion, Sparks does not disclose a method for making a cell-matrix construct for use as a heart valve that involves implanting into an animal a fibrous matrix formed of a polymer matrix that has been seeded with specific selected cells. The Examiner cannot separate the features disclosed in Sparks. The reference must be considered as a whole. Sparks discloses a method of forming a heart valve graft that involves implanting a perforated stainless steel die made up of two members (40 and 42), separated by a cavity within which the valve graft is formed by connective tissue entering the die cavity. This is in fact claimed in Sparks. The stainless steel die is essential to form the three dimensional structure of the heart valve (please see the Sparks, Figures 6-12). There is no similarity whatsoever between the two methods. The only similarity between Sparks and the claims is the name of the resultant product. There is no similarity in the process of making, or the materials used, therefore it is expected that there would be no similar physical properties. Further, Sparks does not disclose seeding any cells in valve grafts. Sparks is very clear when cell seeding is required. Sparks is silent about seeding cells in valve grafts.

The Examiner has cited to Mikos, alleging a disclosure by Vacanti, et al, 1988, that the scaffold should mimic the natural tissue counterpart, and that Vacanti, et al. provides evidence that better results are obtained when the matrix is first implanted, prevascularized and then seeded with select cell, attaching the relevant portion of Mikos (Mikos column 2, lines 16-44).

Vacanti, et al., *J. Pediatric Surgery* 23(1):3-9 (1988) ("Vacanti 1", a copy of which is attached to the evidence appendix) discloses **cell** transplantation by a method which includes attaching cell preparations to bioerodible artificial polymers in cell culture, and then implanting this polymer-cell scaffold into animals. Vacanti 1 discloses a method that allows implantation of large numbers of cells (see Vacanti 1, page 7, right column). Vacanti 1 is not concerned with making tissue grafts, or heart valves, and does not disclose or suggest how they can be made to withstand stress and strain. It is not clear what Mikos means by "better results" cited by the Examiner; however, since Mikos is referring to Vacanti, the "better results" obtained are not concerned with a graft that can withstand repeated stress and strain, since Vacanti is only concerned with enhancing viability of large numbers of transplanted cells seeded onto a fibrous support structure (a gauze). This is supported by a lecture presented by the same author (Vacanti, Beyond *Transplantation*, 123:5459 (1988) ("Vacanti 2" a copy of which is attached to the Evidence appendix), cited by Mikos in the column to which the Examiner referred. Vacanti 2 discussed strides made in cell transplantation, much of the discussion including results from the studies in Vacanti 1. However, Vacanti 2 noted (on page 549, right column), that much work needed to be done, but the hope was that some day cellular chimeras would provide replacement tissue for patients as an alternative to organ transplantation as currently practiced. It is clear from the discussion in Vacanti 1 and 2, that Vacanti 1 cannot make obvious the claimed method, which provides heart valves which can withstand repeated stress and strain. Neither Vacanti recognizes the problem with somehow providing the cell matrix with mechanical properties such as strength, flexibility, resistance to strain – features essential for a structure which will be opened, closed, and subjected to pressures ever second, every hour, every day, every month, every year in the individual's life following implantation. There are simply no comparable requirements when it comes to a parenchymal tissue such as a liver or pancreas.

The Examiner also asserts that the materials used by Appellants are well known and known equivalents are taught by Jauregui or Tang, stating that Tang teaches that bioresorbable materials play a critical role in fabrication of devices used for tissue regeneration. The issue here is not merely using biodegradable materials to make heart valves. That biodegradable materials can be used in tissue engineering is known in the art as correctly stated by the Examiner. Also, that these polymers can be molded to mimic the shape of the tissue to be engineered is also known in the art. However, making a structure with the requisite mechanical physical and mechanical properties necessary for biological function has been the challenge.

The claims do not merely define a method that can be accomplished by substituting the Dacron Mesh in the stainless steel die of Sparks, with the biodegradable materials disclosed in Jauregui or Tang, or the use of the materials disclosed in Jauregui or Tang shaped into a three dimensional structure as suggested by Griffith and or Mikos. Claim 1 requires the cell-matrix be able to withstand repeated stress and strain. This is a critical limitation of a claim to a construct which is to be used to replace a heart valve or heart leaflet, structure which must open and close hundreds of times every hour, thousands of times every day, for years.

According to the Examiner's assertion, the device of Sparks as modified would inherently possess the properties that would be capable of withstanding cyclic stresses and strains, since the valve is designed to function as a replacement of a natural valve. The Examiner must provide a technical reason for a conclusion of inherency. According to the MPEP §2112 "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51

(Fed. Cir. 1999) (citations omitted)". As demonstrated by Shinoka, et al. *Circulation*, 94(9 Suppl):II164-8 (1996) ("Shinoka 1"; abstract attached to Evidence Appendix), the property of withstanding stress and strain is not inherent in biodegradable polymers, therefore a mere disclosure that a biodegradable polymer can be used for tissue engineering cannot make obvious the claimed method and cell-construct. Moreover, it is not clear to the Appellants what modification the Examiner is referring to. If the Examiner is referring to the Dacron mesh, there is no disclosure in Sparks or any reason for a skilled artisan to conclude from Sparks, that the Dacron mesh seeded with cells with have the necessary strength and flexibility and be able to withstand repeated stress and strain. None of the cited references disclose that the biodegradable polymers necessarily possess the ability to withstand repeated stress and strain or how to adapt these polymers to have the requisite property. In fact, Shinoka 1 demonstrates this point.

There is no motivation to combine these references as the Examiner has done, nor would one skilled in the art have a reasonable expectation of success if one did so, based on the art, to yield a structure which can withstand repeated stress and strain. For example, Sparks describes dies containing stainless steel, screws, and plates (see column 5, lines 18-31). This is completely different from the formation of tissue by injecting a cell-polymeric **solution** that gels *in vivo* (Griffith). As stated by the Examiner, Griffith teaches that the degradable template may be shaped or formed prior to implantation into the patient, and as such a combination of Griffith with Sparks would be impossible. Sparks needs the die to be implanted in order to shape the heart valve. Griffith teaches the implantation of a degradable hydrogel template with essentially no defined shape or structure and minimal mechanical properties. Not only is there no motivation to combine, there would be no reasonable expectation of success if one did so.

Mikos discloses preparing biocompatible porous polymer membranes by dispersing particles in a biocompatible polymer solution. There would be no motivation for one of ordinary

skill in the art to replace the Dacron mesh with an absorbable matrix as taught by Mikos or Griffith, nor would one have a reasonable expectation that one could make a strong, flexible structure that could function has a heart valve or leaflet.

Jauregui discloses growing cells *on* a device that is to be implanted. Jauregui does not disclose seeding cells into a fibrous cell structure which is eventually replaced by the cells. Jauregui does not lead one skilled in the art to make a construct that is strong, flexible and useful as a heart valve. A skilled artisan would not be motivated to combine Jauregui and Sparks to arrive at the claimed method and construct, much less have a reasonable expectation of success. According to the MPEP §2143.01 "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the **desirability** of the combination".

Claim 3

Claim 3 is dependent on claim 1 and requires the added limitation that the matrix is first cultured at a site in a patient prior to being transplanted to a second site. Sparks does not disclose or suggest a method of making a cell-matrix construct for use as a heart valve comprising implanting into an animal a cell-matrix construct as recited in claim 1, which is first cultured at a first site in a patient prior to being transplanted to a second site. None of Mikos or Griffith or Jauregui makes up for this deficiency. The references alone or in combination do not recite all the elements of claim 3, and cannot make the claim obvious.

Claim 8

Claim is dependent on claim 1, and recites the added limitation that the heart valve has mechanical strength, and flexibility or pliability. As already disclosed, Sparks does not disclose a method of making a heart valve as recited in claim 1, such that the valve has mechanical strength and flexibility or pliability. The Examiner asserts that the newly formed heart tissue

would inherently possess the strength, flexibility and/or pliability of the tissue it is to replace. The Examiner has provided no technical reasoning for this conclusion and the literature rebuts such a conclusion. Without such a disclosure in Sparks, it would appear that the Examiner is concluding that because the graft is intended to replace heart valve, it would have all the characteristics of a heart valve. Please see Shinoka, et al. *Ann Thorac Surg.*, 60(6 Suppl):S513-6 (1995) ("Shinoka 2", a copy of abstract attached to Evidence Appendix) which discussed the disadvantages of valve replacements using other materials such as bioprosthetics or mechanical valves. Clearly, the mere fact that an object is intended to replace a biological structure does not inherently confer to it the properties of that structure. The references alone or in combination do not recite all the elements of claim 8, and cannot make the claim obvious.

Claims 9 and 10

Sparks does not suggest or disclose a method of making a cell-matrix construct for use as a heart valve, wherein the cell matrix construct is formed of the polymer selected form the polymers listed in claims 9 and 10, wherein the cell-matrix construct can withstand repeated stress and strain. As previously discussed, these polymers do not inherently possess the ability to withstand repeated stress and strain (Shinoka 1); Sparks does not contemplate making cell constructs with this property and none of Mikos or Griffith or Jauregui or Tang make up for this deficiency. The references alone or in combination do not recite all the elements of claims 9 and 10, and cannot make the claims obvious.

Claim 11

Claim 11 is dependent on claim 1, and requires the added limitation that the cell-matrix construct contain interconnected pores in the range of between approximately 100 and 300 microns. Sparks does not disclose a pore size range for the polymer matrix or recognize the importance of a specific pore size and does not disclose the pore size range recited in claim 11

for the production of heart valves or valve leaflets. None of the secondary references make up for this deficiency. The references alone or in combination do not recite all the elements of claim 11, and cannot make the claim obvious.

Claims 12 and 13

Sparks does not disclose or suggest a method for making a cell construct for use as a heart valve or valve leaflet, wherein the construct can withstand repeated stress and strain, and wherein the construct includes growth factors as recited in claims 12 and 13. The references alone or in combination do not recite all the elements of claim 12 and 13, and cannot make the claims obvious.

Claim 14

None of the references, either alone or in combination, disclose a method of making a cell-matrix construct for use as a heart valve or valve leaflet, wherein the construct can withstand repeated stress and strain and wherein the cell-matrix further comprises bioactive factors incorporated to between 1 and 30% by weight (please see the specification at column 6, lines 4-13). Sparks is silent about adding bioactive factors. Mikos and Griffith do not disclose this dosage range. The references cannot make obvious claim 14.

Claim 15

Claim 15 defines the cell construct of any one of claims 1-14. Sparks discloses a method of making heart valves or leaflets that would result in a cell construct made of Dacron mesh encapsulated by connective tissue (Sparks, column 5, lines 32-40) in a stainless steel die. No disclosure of having flexibility or the necessary mechanical strength. This is very different from the construct defined by any of claims 1-14, which is made of a fibrous matrix formed of a biocompatible, biodegradable polymer having the cells listed in claim 1 seeded therein, and which can withstand repeated stress and strain. Sparks does not contemplate making a construct

that can withstand repeated stress and strain and does not disclose how to accomplish this. None of Mikos or Griffith or Jauregui or Tang makes up for this deficieney. Therefore claim 15 is not obvious over the cited prior art.

(8) Conclusion

The cited art does not disclose each claimed limitation, the motivation to combine as appellants have done, nor would they alone or in combination lead one skilled in the art to have a reasonable expectation that such a combination would be useful as a heart value or leaflet, having the requisite flexibility and mechanical properties.

Allowance of claims 1-5 and 8-15, is respectfully solicited.

Respectfully submitted,

/Patrea L. Pabst/
Patrea L. Pabst
Reg. No. 31,284

Date: December 8, 2006

PABST PATENT GROUP LLP
400 Colony Square, Suite 1200
1201 Peachtree Street
Atlanta, Georgia 30361
(404) 879-2151
(404) 879-2160 (Facsimile)

Claims Appendix: Claims On Appeal

1. A method for making a cell-matrix construct for use as a heart valve comprising

implanting into an animal a cell-matrix construct comprising a fibrous matrix in the shape of a heart valve or heart valve leaflet, wherein the matrix is formed of a biocompatible, biodegradable polymer having seeded therein cells selected from the group consisting of endothelial cells, myofibroblasts, skeletal muscle cells, vascular smooth muscle cells, myocytes, fibromyoblasts, and ectodermal cells, wherein the cell-matrix construct can withstand repeated stress and strain.

2. The method of claim 1 wherein the matrix is seeded with dissociated parenchymal or connective tissue cells.

3. The method of claim 1 wherein the matrix is first cultured at a first site in a patient prior to being transplanted to a second site.

4. The method of claim 1 wherein the matrix is in the form of a heart valve leaflet.

5. The method of claim 1 wherein the cell-matrix construct is seeded with vascular smooth muscle cells and endothelial cells and implanted to form a heart valve.

8. The method of claim 5 wherein the heart valve has mechanical strength, and flexibility or pliability.

9. The method of claim 1 wherein the cell-matrix construct is formed of a polymer selected from the group consisting of poly(lactide) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates, and degradable polyurethanes.

10. The method of claim 1 wherein the cell-matrix construct is formed of a polymer selected from the group consisting of polyacrylates, ethylene-vinyl acetate polymers, acyl substituted cellulose acetates, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, and nylon.

11. The method of claim 1 wherein the cell-matrix construct contains interconnected pores in the range of between approximately 100 and 300 microns.

12. The method of claim 1 wherein the cell-matrix construct includes growth factors.

13. The method of claim 12 wherein the growth factors are selected from the group consisting of heparin binding growth factor (hbgf), transforming growth factor alpha or beta (TGF), alpha fibroblastic growth factor (FGF), epidermal growth factor (TGF), vascular endothelium growth factor (VEGF), insulin, glucagon, estrogen, nerve growth factor (NGF) and muscle morphogenic factor (MMP).

14. The method of claim 1 wherein the cell-matrix further comprises bioactive factors incorporated to between one and 30% by weight.

15. The cell-matrix construct of any one of claims 1-14.

16. A cell-matrix construct for use as a heart valve or heart valve leaflet comprising a fibrous polymeric matrix in the shape of a heart valve or heart valve leaflet, wherein the matrix is formed of a biocompatible, biodegradable polymer having seeded thereon cells comprising myofibroblasts grown to confluence and then endothelial cells seeded thereon.

17. The cell-matrix construct of claim 16 wherein the cell-matrix construct can withstand repeated stress and strain.

Evidence Appendix

Evidence submitted with the Amendment and Response filed on October 3, 2005

Shinoka, et al. *Circulation*, 94(9 Suppl):II164-8 (1996)

Shinoka, et al. *Ann Thorac Surg.*, 60(6 Suppl):S513-6 (1995)

Reference submitted with the information disclosure statement filed on February 19, 2004 and relied upon by the Examiner in the office action mailed on June 8, 2006.

Vacanti, et al., J. Pediatric Surgery 23(1):3-9 (1988).

Reference submitted with the information disclosure statement filed on February 19, 2004

Vacanti, Beyond *Transplantation*, 123:5459 (1988)

1: Circulation. 1996 Nov 1;94(9 Suppl):II164-8. Related Articles Links

Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model.

Shinoka T, Ma PX, Shum-Tim D, Breuer CK, Cusick RA, Zund G, Langer R, Vacanti JP, Mayer JE Jr.

Department of Cardiovascular Surgery, Children's Hospital, Boston, Mass, USA.

BACKGROUND: We have previously reported the successful creation of tissue-engineered valve leaflets and the implantation of these autologous tissue leaflets in the pulmonary valve position. This study was designed to trace cultured cells that were seeded onto a biodegradable polymer with the use of a 1,1'-dioctadecyl-3,3,3',3'-tetramethylindo-carbocyanine perchlorate (Di-1) cell-labeling method. We also examined the time-related biochemical, biomechanical, and histological characteristics and evolution of these tissue constructs. METHODS AND RESULTS: Mixed cell populations of endothelial cells and fibroblasts were isolated from explanted ovine arteries. Endothelial cells were selectively labeled with an acetylated low density lipoprotein marker and separated from fibroblasts with the use of a fluorescence-activated cell sorter. A synthetic biodegradable scaffold consisting of polyglycolic acid fibers was seeded first with fibroblasts, then coated with endothelial cells. Using these methods, we implanted autologous cell/polymer constructs in six animals. In two additional control animals, a leaflet of polymer was implanted without prior cell seeding. In each animal, cardiopulmonary bypass was used to completely resect the right posterior leaflet of the pulmonary valve and replace it with an engineered valve leaflet with (n = 6) or without (n = 2) prior cultured cell seeding. The animals were killed either after 6 hours or after 1, 6, 7, 9, or 11 weeks, and the implanted valve leaflets were examined histologically, biochemically, and biomechanically. 4-Hydroxyproline assays were performed to determine collagen content. Leaflet strength was evaluated in

1: Ann Thorac Surg. 1995 Dec;60(6 Suppl):S513-6.

Tissue engineering heart valves: valve leaflet replacement study in a lamb model.

Shinoka T, Breuer CK, Tanel RE, Zund G, Miura T, Ma PX, Langer R, Vacanti JP, Mayer JE Jr.

Department of Cardiovascular Surgery, Children's Hospital, Boston, MA 02115, USA.

BACKGROUND. Valve replacements using either bioprosthetic or mechanical valves have the disadvantage that these structures are unable to grow, repair, or remodel and are both thrombogenic and susceptible to infection. These characteristics have significantly limited their durability and longevity. In an attempt to begin to overcome these shortcomings, we have tested the feasibility of constructing heart valve leaflets in lambs by seeding a synthetic polyglycolic acid fiber matrix in vitro with fibroblasts and endothelial cells. METHODS. Mixed cell populations of endothelial cells and fibroblasts were isolated from explanted ovine arteries. Endothelial cells were selectively labeled with an acetylated low-density lipoprotein marker and separated from the fibroblasts using a fluorescent activated cell sorter. A synthetic biodegradable scaffold constructed from polyglycolic acid fibers was seeded with fibroblasts, which grew to form a tissue-like sheet. This tissue was subsequently seeded with endothelial cells, which formed a cellular monolayer coating around the leaflet. Using these constructs, autologous (n = 3) and allogenic (n = 4) tissue engineered leaflets were implanted in 7 animals. In each animal the right posterior leaflet of the pulmonary valve was resected and replaced with an engineered valve leaflet. RESULTS. All animals survived the procedure. Postoperative echocardiography demonstrated no evidence of stenosis and trivial pulmonary regurgitation in the autografts and moderate regurgitation in the allogenic valves. Collagen analysis of the constructs showed development of an extracellular matrix. Histologic evaluation of the constructs demonstrated

appropriate cellular architecture. CONCLUSIONS. This preliminary experiment showed that a tissue engineered valve leaflet constructed from its cellular components can function in the pulmonary valve position. Tissue engineering of a heart valve leaflet is feasible, and these preliminary studies suggest that autograft tissue will probably be superior to allogenic tissue.

MeSH Terms:

- Animals
- Bioprosthesis*
- Culture Techniques*
- Endothelium, Vascular/cytology
- Fibroblasts/cytology
- Heart Valve Prosthesis
- Heart Valves*/surgery
- Polyglycolic Acid
- Sheep

Substances:

- Polyglycolic Acid

PMID: 8604922 [PubMed - indexed for MEDLINE]

Selective Cell Transplantation Using Bioabsorbable Artificial Polymers as Matrices

By Joseph P. Vacanti, Martin A. Morse, W. Mark Saltzman, Abraham J. Domb, Antonio Perez-Atayde, and Robert Langer

Boston and Cambridge, Massachusetts

● To date, selective cell transplantation has involved injecting cell suspensions into tissues or the vascular system. This study describes attaching cell preparations to bioerodable artificial polymers in cell culture and then implanting this polymer-cell scaffold into animals. Using standard techniques of cell harvest, single cells and clusters of fetal and adult rat and mouse hepatocytes, pancreatic islet cells, and small intestinal cells have been seeded onto biodegradable polymers of polyglactin 910, polyanhydrides, and polyorthoester. Sixty-five fetuses and 14 adult animals served as donors. One hundred fifteen polymer scaffolds were implanted into 70 recipient animals: 66 seeded with hepatocytes; 23 with intestinal cells and clusters; and 26 with pancreatic islet preparations. The cells remained viable in culture, and in the case of fetal intestine and fetal hepatocytes, appeared to proliferate while on the polymer. After four days in culture, the cell-polymer scaffolds were implanted into host animals, either in the omentum, the interscapular fat pad, or the mesentery. In three cases of fetal intestinal implantation coupled with partial hepatectomy, successful engraftment occurred in the omentum, one forming a visible 6.0 mm cyst. Three cases of hepatocyte implantation, one using adult cells and two using fetal cells, have also engrafted, showing viability of hepatocytes, mitotic figures, and vascularization of the cell mass. To date, no pancreatic islets have survived implantation. This method of cell transplantation, which we have termed "chimeric neomorphogenesis," is an alternative to current methods and requires further study.

INDEX WORDS: Transplantation; cell culture; fetal cell transplantation.

SELECTIVE cell transplantation of only those parenchymal elements necessary to replace lost function has been studied as an alternative to whole or partial organ transplantation.[1] In the past, cells have been harvested, dispersed into a suspension, and then innoculated into various tissues. We report attaching parenchymal cells from liver, intestine, and pancreas onto biocrodable artificial polymers and then implanting these polymer-cell scaffolds into animals as a novel method of cell transplantation.

MATERIALS AND METHODS

Polymers

Three synthetic absorbable polymers have been used to fabricate filaments and discs as matrices for cell attachment, growth, and implantation (Fig 1).

Polyglactin. This polymer was developed as absorbable synthetic suture material. It is a 90-10 copolymer of glycolide and lactide and is produced as Vicryl braided absorbable suture (Ethicon Co, Somerville, NJ).[2]

Polyorthoesters. The specific polymer used was 3,9-bis(ethylidene-2,4,8,10-tetraoxaspiro[5.5]) undecane copolymer with *trans*-1,4-cyclohexanedimethanol and 1,6-hexandiol in a molar ratio 2:1:1, respectively (SRI, CA).[3]

Polyanhydride. The specific polymer used was a polyanhydride of bis(1,4-carboxyphenoxy) propane and sebacic acid. It is biocompatible and has been used extensively in drug delivery applications.[2,4]

Polymer Configuration

Small wafer discs or filaments of polyanhydrides and polyorthoesters were fabricated using one of the following methods.

Solvent casting. A solution of the polymer (10% in methylene chloride) was cast on a branching pattern relief structure as a disc 10 mm in diameter for 10 minutes at 25°C using a Carver press. After solvent evaporation, a film 0.5 mm in thickness with an engraved branching pattern on its surface was obtained.

Compression molding. One hundred mg of the polymer was pressed (30,000 psi) into a branching pattern relief structure 10 mm in diameter, 0.5 mm discs were obtained.

Filament drawing. Filaments were drawn from the molten polymer (30 μm in diameter). Small flattened 1.0 cm tufts were used for the experiments.

Polyglactin 910. Multiple fibers of 90-10 copolymer of glycolide and lactide converging to a common base were fashioned from suture material of 0-Vicryl by fraying the braided end of the polymer (Fig 2). These branching fiber clusters were approximately 1.0 cm in height. The individual fibrils were 30 μm in diameter.

Animals

Young adult and fetal Sprague Dawley rats and C57 Bl/6 mice (Charles River Labs, Wilmington, MA) were used as cell donors for all experiments. The animals were housed individually, allowed access to food and water ad lib, and maintained at 12 hour light and dark intervals. Anesthesia was obtained with an intraperitoneal injection of pentobarbital (Abbott Labs, North Chicago, IL) at a dose of 0.05 mg/g and supplemented with methoxyflurane (Pitman-

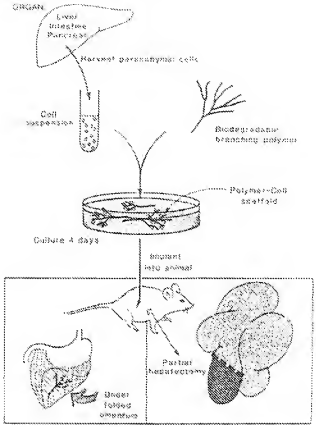
From the Departments of Surgery and Pathology, The Children's Hospital and the Harvard Medical School, Boston, and the Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge.

Supported by the March of Dimes Grant No. 5-543, Basil O'Connor Starter Research Grant, and NIH Grant No. 6M 26698.

Presented at the 18th Annual Meeting of the American Pediatric Surgical Association, Hilton Head Island, South Carolina, May 6-9, 1987.

Address reprint requests to Joseph P. Vacanti, MD, The Children's Hospital, 300 Longwood Ave, Fegan Building, Fifth Floor, Boston, MA 02115.

0022-3468/88/2301-0002$03.00/0



in the midline using sterile technique. The common bile duct was isolated, and the pancreas visualized. Two and a half milliliters of 2.0% type II collagenase was infused into the pancreas by injection into the common bile duct using the technique described by Gotoh et al.[6] After five minutes, the pancreas was transferred to a sterile hood for islet cell isolation. Briefly, the tissue was placed into a 25% Ficoll

the interscapular fat pad; (2) the omentum; and (3) the bowel mesentery (Fig 3).

Most animals underwent a partial hepatectomy to stimulate cell growth. Animals were sacrificed at day 3, 7, or 14 and the implants were examined histologically with hematoxylin and eosin. Polymers without cells served as controls. Polymer-cell scaffolds were exam-

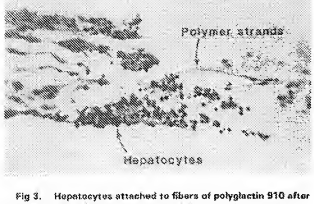


Fig 3. Hepatocytes attached to fibers of polyglactin 910 after four days in culture. Cells very from healthy to degenerated and necrotic (Hematoxylin and eosin, original magnification ×172).

ined histologically after four days in culture and before implantation to assess cell attachment and viability.

RESULTS

Seventy-nine animals, including 14 adults and 65 fetuses, were used as donors for cell harvest; 115 polymer scaffolds were prepared for implantation. Sixty-six of these scaffolds were seeded with hepatocytes, 23 with intestinal cells and clusters, and 26 with pancreatic islets and cell preparations. Implantation was performed in 70 recipient animals. Fifty-eight were killed at seven days for histologic examination of the implant while three were examined at three days, and nine at 14 days after implantation.

Cell viability on the polymer scaffold at 3 to 4 days in culture varied with the type of polymer material used. Less than 10% of the cells were viable on the polyanhydride discs, whereas 80% of cells cultured on polyorthoester discs and filaments remained viable, and over 90% survived on polyglactin 910 (Fig 3).

Blood vessel ingrowth was noted three days after implantation with all of the polymer types and configurations. In the implanted fiber networks, new blood vessels formed in the interstices between the polymer filaments. The polymer discs showed capillary formation immediately adjacent to the polymer material. This angiogenic response accompanied an inflammatory infiltrate that displayed both an acute phase and a chronic foreign body reaction to the implanted polymers. The intensity of inflammation varied with the polymer type tested: polyanhydride elicited the most severe acute and chronic response although the inflammation surrounding branching fibers of either polyorthoester or polyglactin appeared proportionately greater than the disc configuration because of the greater surface area of exposed foreign material to host.

Histologic examination of liver cell implants in three animals showed evidence of successful engraftment of hepatocytes at seven days. Small clusters of healthy appearing hepatocytes were seen with bile canaliculi between adjacent cell membranes and some areas demonstrated mitotic figures. The cells were surrounded by an inflammatory response and blood vessels coursed around and through the cell clusters. Polymer material was seen immediately adjacent to the cells (Fig 4).

Successful engraftment of intestinal cells and clusters were observed in three animals. Histologic findings were similar to the hepatocyte implants with one exception (Figs 5A-C). On gross examination of the implant at seven days, a cystic structure approximately 6.0 mm in length was found at the implant site (Fig 5A) with polymer fibers splayed within its wall. Microscopic examination revealed well-differentiated intestinal epithelium lining the cavity with mucous and cellular debris within the lumen. One wall of the cyst contained polymer fibers, blood vessels, and inflammatory cells immediately adjacent to the intestinal epithelium (Fig 5B). The other wall included a muscular coating that suggested that the polymer held a small minced piece of fetal intestine as the origin of the cyst that eventually developed. The cyst displayed well-differentiated intestinal epithelium with mucous secreting cells (Fig 5C). Other clusters of intestinal epithelium demonstrated active mitoses.

No viable pancreatic islets or other cell types were found in any of the pancreas implants tested. However, all of the implants were accompanied by an inflammatory and angiogenic response similar to those noted with liver and intestine.

Control polymers implanted without prior cell seed-

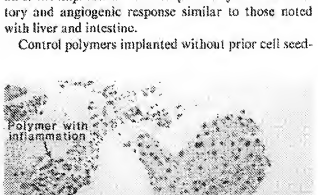


Fig 4. Implant of hepatocytes from adult rat donor into omentum. The polymer-cell implant has been in place for seven days before death. Hepatocytes are healthy and several mitotic figures can be seen. Blood vessels are present in the mass. To the left, an inflammatory infiltrate in the area of the polymer is observed (Hematoxylin and eosin, original magnification, ×172).

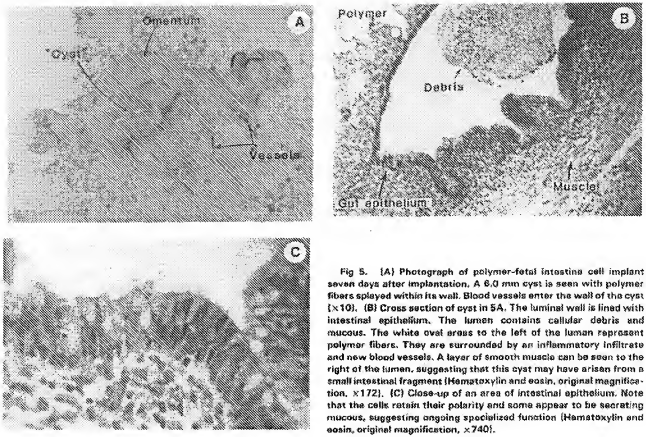


Fig 5. (A) Photograph of polymer-fetal intestine cell implant seven days after implantation. A 6.0 mm cyst is seen with polymer fibers splayed within its wall. Blood vessels enter the wall of the cyst (×10). (B) Cross section of cyst in 5A. The luminal wall is lined with intestinal epithelium. The lumen contains cellular debris and mucous. The white oval areas to the left of the lumen represent polymer fibers. They are surrounded by an inflammatory infiltrate and new blood vessels. A layer of smooth muscle can be seen to the right of the lumen, suggesting that this cyst may have arisen from a small intestinal fragment (Hematoxylin and eosin, original magnification, ×172). (C) Close-up of an area of intestinal epithelium. Note that the cells retain their polarity and some appear to be secreting mucous, suggesting ongoing specialized function (Hematoxylin and eosin, original magnification, ×740).

ing elicited an angiogenic and inflammatory response similar to their counterparts that had been seeded with parenchymal cells and maintained in culture. This suggested that the cells themselves did not play a major role in the inflammation and neovascularization seen.

DISCUSSION

Many diseases of the liver, intestine, and pancreas result in organ failure. This century has seen the development of specific pharmacologic therapy to replace lost function. Insulin replacement for diabetes mellitus is an example.

The emergence of organ transplantation and the science of immunobiology has allowed replacement of the kidney, heart, liver, and other organs. However, as our ability to perform these complex operations has improved, the limitations of the technology have become more evident. For example, in pediatric liver transplantation, donor scarcity has increased as more programs have opened. Only a small number of donors are available in the United States for 800 to 1,000 children in liver failure and those children that undergo transplantation are often so ill by the time a liver is found that the likelihood of success is diminished. The surgery is complex and usually associated with major blood loss. The preservation time is short and, therefore, results in major logistical problems in matching a distant donor with a recipient. For these reasons, the undertaking is expensive and labor intensive, requiring a major investment of resources available only in tertiary care facilities.

Selective cell transplantation of only those parenchymal elements necessary to replace lost function has been proposed as an alternative to whole or partial organ transplantation.[1] It has several attractive features. It avoids major surgery with its attendant blood loss, anesthetic difficulties and complications. It replaces only those cells that supply the needed function and, therefore, problems with passenger leukocytes, antigen presenting cells, and other cell types which may promote the rejection process are avoided. Adding the techniques of cell culture provides another set of tools to aid in the transplantation process. The ability to expand cell numbers with proliferation of cells in culture allows autotransplantation of one's own tissue. Skin equivalents using contracted collagen lattices and epidermal cells have been described.[9,10] Insertion of gene segments, and deletion of antigenic components while the cells are in culture is also possible with current technology.

Islet cell transplantation as an experimental treat-

ment of diabetes mellitus is an area of current research. Although there is evidence of short-term function, long-term results have been less satisfactory.[11,12] Currently, whole organ pancreatic transplantation is the preferred replacement. Hepatocyte injections into the portal circulation have been attempted to support hepatic function. A recent novel approach in which hepatocytes were attached to collagen-coated microcarrier beads prior to injection into the peritoneal cavity demonstrated successful implantation, viability of the implanted hepatocytes, and function. The authors suggested that cell attachment to a matrix prior to implantation was an important component of successful engraftment and function.[13]

Our studies of isolating parenchymal cells, attaching them to biodegradable polymer scaffolds in cell culture, and implanting them into hosts are based on several biologic observations:

(1) Every structure in living organisms is in a dynamic state of equilibrium. It undergoes constant renewal, remodeling, and replacement of functional tissue. The degree of change varies from organ to organ and structure to structure.

(2) Structural cells, if placed in a dissociated state, tend toward reforming structure. Their ability to do so depends on the environment in which they are placed and the degree of alteration they have undergone. Examples include capillary endothelial cells that form tubes in vitro under certain conditions[14] and bile duct cells that form tubes under the proper conditions.[15]

(3) Tissue cannot be implanted in volumes >1.0 to 3.0 μL because nutrition is limited by the maximum diffusion distance until angiogenesis occurs.[16]

(4) Cell shape determined by cytoskeletal components and attachment to matrix plays an important role in cell division and differentiated function.[17,18] If dissociated cells are placed into mature tissue as a suspension without cell attachment, they may have a difficult time finding attachment sites, achieving polarity, and functioning because they begin with intrinsic organization. This may limit the total number of implanted cells that can remain viable to organize, proliferate, and function.

We reasoned that if we provided an organized scaffolding to which the cells were already attached, we could increase the total number of implanted cells. Artificial biodegradable polymers were chosen for several reasons. We could engineer configuration, manageability, tensile strength, and rate of degradation to a great degree with a man-made plastic and also might be able to modify the inflammatory response by modifying the material. The polymer material could be coated with other cell types or with attachment factors to increase cell attachment. In addition, we have considerable experience in placing biologically active molecules such as growth factors directly into the polymer, and allowing slow release of these agents in a controlled and predictable way.[19] Finally, by the use of biodegradable matrices, we provide only a temporary scaffold, which eventually is reabsorbed, leaving structural support of the mass to mesenchymal elements supplied by the host, and modified by the implanted cells. Although some of the polymers studied caused somewhat of an inflammatory response, it is likely that further purification will reduce or eliminate this problem. This has already been shown to be the case with ethylene-vinyl acetate[19] and polyanhydrides.[5]

This technology allowed us to design the polymers to meet the biologic needs of the system we wished to create. The configuration of the polymer scaffold must have enough surface area for the cells to be nourished by diffusion while neovascularization occurs. The new blood vessels must interdigitate with the implanted parenchymal elements to continue to support their growth, organization, and function. Polymer discs seeded with a monolayer of cells and branching fiber networks both satisfy these needs. The branching fibers are based on the same principles that nature has used to solve the problem of increasing surface area proportionate to volume increases. All multicellular organisms utilize this repeating branching structure. Branching systems represent communication networks between organs as well as the functional units of individual organs.[20] Seeding this configuration with cells and implanting the structure as fibers allows us to implant large numbers of cells, each of which is exposed to the environment of the host. Therefore, free exchange of nutrients and waste can occur while neovascularization is achieved. If functional, the new three-dimensional mass would be a true chimera of parenchymal elements of the donor, and mesenchymal elements of the recipient. The term "chimeric neomorphogenesis" describes the process that has occurred.

The results of this study demonstrate that cells from liver, intestine, and pancreas can be successfully harvested, and will attach to artificial biodegradable polymers. They will survive in culture in this configuration and can then be implanted into a host in a variety of locations. An inflammatory response that is mediated by both the wound and the nature of the polymer will occur. Successful engraftment of small clusters of hepatocytes and intestinal cells has been demonstrated. However, we do not yet have evidence of cell function in the new environment. Further studies to define the optimal characteristics of the polymer, attachment parameters, growth criteria, and function, need to be performed. Clinical application in diabetes with beta cells of the pancreas, in hepatic failure with

hepatocytes and biliary cells, and in intestinal insufficiency with intestinal epithelium remains the long-term goal.

ACKNOWLEDGMENT

We wish to express our sincere gratitude to Judah Folkman for advice, Dr Jorge Heller for his generous gift of polyorthoester, Deborah Stark for her technical assistance, and Susann Antezak for preparation of this manuscript.

REFERENCES

1. Russell PS: Selective transplantation. Ann Surg 201:255-262, 1985

2. Craig PH, Williams JA, Davis KW, et al: A biological comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures. Surgery 141:1-10, 1975

3. Heller J, Penhale WH, Helwing RF, et al: Release of norethindrone from polyacetals and polyorthoesters. AIChE Symposium Series, 206:28-36, 1981

4. Leong KW, D'Amore P, Marletta M, et al: Bioerodable polyanhydrides as drug carrier matrices II. Biocompatability and chemical reactivity. J Biomed Mat Res 20:51, 1986

5. Domb AJ, Langer R: Polyanhydrides I. Preparation of high molecular weight polyanhydrides. J Poly Sci (in press)

6. Kopecek J, Ulbrich K: Biodegradation of biomedical polymers. Prog Poly Sci 9:1, 1983

7. Salgen PO: Preparation of rat liver cells. III. Enzymatic requirements of tissue dispersion. Exp Cell Res 82:391-398, 1973

8. Gotoh M, Maki T, Kiyozumi T, et al: An improved method of isolation of mouse pancreatic islets. Trans 40:437-438, 1985

9. Bell E, Ehrlich HP, Buttle DJ, et al: Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science 211:1052-1054, 1981

10. Gallico GG, O'Connor NE, Compton CC, et al: Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 311:448-451, 1984

11. Sutherland DER: Pancreas and islet transplantation. I. Experimental studies. Diabetologia 20:161-185, 1981

12. Sutherland DER: Pancreas and Islet Transplantation. II. Clinical Trials. Diabetologia 20:435-450, 1981

13. Demetriou AA, Whiting JF, Feldman D, et al: Replacement of liver function in rats by transplantation of microcarrier-attached hepatocytes. Science 233:1190-1192, 1986

14. Folkman J, Haudenschild C: Angiogenesis in vitro. Nature 288:551-556, 1980

15. Vacanti JP, Morse MA, Haudenschild C, et al: The isolation and growth of biliary epithelial cells in long-term monolayer cell culture. (Submitted)

16. Folkman J, Hochberg MM: Self-regulation of growth in three dimensions. J Exp Med 138:745-753, 1973

17. Folkman J, Moscona A: Role of cell shape in growth control. Nature 273:345-349, 1978

18. Ben-Ze'ev A, Farmer SR, Penman S: Protein synthesis requires cell-surface contact while nuclear events respond to cell shape in anchorage-dependent fibroblasts. Cell 2:365-372, 1980

19. Langer R, Folkman J: Polymers for the sustained release of proteins and other macromolecules. Nature 263:797-800, 1976

20. MacDonald N: Trees and Networks in Biological Models. New York, Wiley, 1983

Discussion

P. Donahoe (Boston): This interesting approach, which uses biodegradable materials as an artificial matrix, has been successfully done in two-dimensional systems by Drs John Burke and Howard Green for the replacement of skin for major burns. Dr Vacanti is attempting this technique in a three-dimensional system. I look forward to further screening of matrix materials in the future. After maintaining dispersed cells from liver, pancreas, and intestine in culture for three days before implantation, small viable implants were found in three of 66 hepatic implants, three of 26 small intestinal implants with a 6 mm cystic structure, and 0 of 23 pancreatic implants reflecting also our own nonsuccess with that particular organ. Though falling far short of providing needed organs for transplantation, which is the author's dream, it does provide us with some promising techniques. I would make a number of suggestions for improving the rate of graft survival. First of all, allogeneic grafts were used and rejection can expect to be high. I would suggest using syngeneic mice to test the hypothesis more strongly. Ontogeny studies should be done in order to determine at which fetal age growth would be allowed. The position of implantation is also vital. We too lost a high percentage of grafts in omentum and in mesentery, as well as the hepatic capsule and in the retroperitoneum, so we turned to the subrenal capsule where structures can grow without being ejected. Can the authors tell us why the polyorthoester was so unsuccessful? Were implantation toxicity studies done before undertaking the present studies? What will be your next experimental designs? We will follow subsequent studies with great interest.

J.P. Vacanti (closing): Syngeneic mice eliminate rejection as a potential mediator of the inflammatory response, which has been significant in some of the polymers we have tested. The time-line studies with different aged animals are very important and I appreciate that point. Our hope is that adult cells can be used with success as can fetal cells. We have evolved the idea that terminal differentiation of cells is probably not very common and that in the appropriate circumstance with the appropriate communication with other cells and matrix, cells can be manipulated and can express genes that they do not usually express. We have come to the conclusion that the matrix component of different tissues probably contributes to the successful engraftment of different cell types. So

which particular location for which particular cell type needs to be worked out. We wonder whether we can take different polymers for which we have a long experience as slow release reservoirs for bioactive molecules and then use them to precondition different tissues with various growth factors and differentiation factors before we do the implant. We might be able to fool the tissue into thinking it is fetal tissue as opposed to adult tissue. The polyanhydrides at the configuration that we used were the least successful and the most toxic and they are very acidic, and it is very difficult to keep up with the acid release in culture and probably in in vivo. More work needs to be done with this particular polymer family. The polyorthoester is reasonably noninflammatory and provided good cell attachment.

Beyond Transplantation

Third Annual Samuel Jason Mixter Lecture

Joseph P. Vacanti, MD

It is an honor for me to present the Third Annual Samuel Jason Mixter Lecture before this distinguished audience. The subject of this report is the field of transplantation in its broadest definition: what has been achieved, and what may lie ahead. It is appropriate that this summary be presented in this forum, since many of the creative insights and innovations have come from New England surgeons. I will focus on a few of these contributions, but this in no way is intended to diminish the impact of the efforts of many others, both here in New England as well as in other centers in the United States and abroad.

In simple terms, surgeons treat patients by taking things out, putting things in, or moving things around. Organ transplantation is an extreme form of reconstructive surgery, and involves replacing lost function by putting something in. After years of careful experimental work, first in the laboratory and then in the clinical setting, surgeons and physicians at the Peter Bent Brigham Hospital, Boston, were poised to undertake organ transplantation in the mid-1950s. Joseph Murray, MD (Fig 1), your current president, headed the team that performed the first successful organ transplant in 1954.[1] It was a kidney, transplanted from an identical twin into his severely ill brother. The immunologic barrier of placing tissue from one nongenetically identical individual into another was overcome again at the Peter Bent Brigham Hospital under Dr Murray's direction in the early 1960s. These sentinel achievements marked a new era in medicine and surgery, as well as created new fields of scientific investigation such as transplantation biology and immunology. One of the very important lessons to learn from these events is that they were not isolated occurrences of good fortune, but rather the culmination of years of thoughtful research in the laboratory and an approach to treatment of patients in a new way because of dissatisfaction with current methods.

Renal transplantation and bone marrow transplantation evolved rapidly over the next 25 years, but the transplantation of other organs remained largely experimental until the late 1970s. With the advent of better immunosuppression, standardized surgical and anesthetic techniques, and the ability to monitor and control rejection, extrarenal organ transplantation underwent explosive growth. At the end of 1986, there were almost 100 heart transplantation centers, 41 liver transplantation centers, and 27 pancreatic transplant centers. Cumulatively, over 2000 liver transplants, over 3000 heart transplants, and almost 500 pancreas transplants had been performed by the end of 1986 in the United States (Fig 2). The Boston Center for Liver

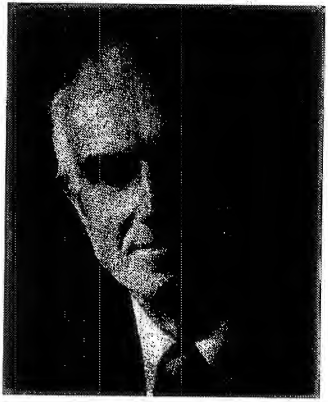


Fig 1.—Joseph E. Murray, MD, Peter Bent Brigham Hospital, Boston, surgeon who performed world's first successful organ transplant in 1954, that of a kidney into an identical twin.

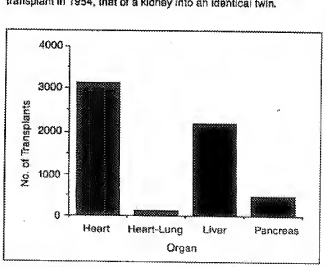


Fig 2.—Cumulative number of extrarenal transplants performed in United States by December 1986.

Accepted for publication Feb 23, 1988.

From the Departments of Surgery, Harvard Medical School and The Children's Hospital, Boston.

Read before the Annual Meeting of the New England Surgical Society, Bretton Woods, NH, Sept 12, 1987.

Reprint requests to The Children's Hospital, 300 Longwood Ave, Boston, MA 02115 (Dr Vacanti).

Transplantation was created in January 1964 and was the sixth center to offer liver transplantation; now, as I mentioned, there are over 40 such centers.[2]

These achievements are truly remarkable, and the life that is returned to patients at times seems miraculous. However, the practice of transplantation is not without problems. I would like to share with you the experience of pediatric liver transplantation as we currently must practice it in the Boston area. The patient shown in Fig 3 was cared for by our program in 1986. This photograph was

Fig 3.—Infant with marked cirrhosis from biliary atresia, leading to portal hypertension, malnutrition, gastrointestinal tract bleeding, and sepsis.

obtained while we continued to treat him as an outpatient. We make every effort to avoid hospitalization to prevent colonization by hospital-acquired resistant organisms and to keep the children with their families. We had been searching nationally for an appropriate donor organ for several months as the patient's condition progressively deteriorated. His course was complicated by marked malnutrition, portal hypertension, episodes of gastrointestinal tract bleeding, and systemic sepsis. Five days after transplantation, he died of overwhelming sepsis from a hospital-acquired organism. His condition was a direct result of the critical donor organ shortage among small children. Between 12% and 40% of infants will die before liver transplantation for lack of a donor, and many others will become so critically ill that the success of transplantation is diminished.[3] Because of the donor scarcity and the short preservation times currently allowable for a liver, teams must simultaneously harvest donor organs from as far away as 2000 miles. To accomplish this successfully, private jet aircraft are needed to shorten transportation time

Fig 4.—Chartered aircraft for organ retrieval.

Fig 5.—Left, Paul S. Russell, MD, chief of transplantation at Massachusetts General Hospital, Boston. Right, John F. Burke, MD, chief of trauma services at Massachusetts General Hospital and creator of artificial dermis for massively burned patients.

(Fig 4). Often, we also employ helicopters to fly to the airport, to the donor hospital, and then back to the hospital to minimize preservation time. This need adds enormous cost and logistical complexity to the operative effort, thereby increasing the risk.

Small children require tiny livers, often from newborn donors. The vascular and biliary anastomoses are difficult, again increasing the risk of great failure in the absence of artificial hepatic support. However, despite the many limitations, we all recognize that transplantation does work. It does save lives. The critical issue we must face is whether organ transplantation realistically can ever match the need. As an example, liver disease accounts for 30 000 deaths per year in the United States, with an estimated annual cost of $14 billion to the US health care system.[4] Ten million patients in the United States suffer from diabetes mellitus, of whom 600 000 are insulin dependent. Thirty percent of these patients have the devastating complications of diabetes. In 1984 dollars, their care resulted in a cost of $13.75 billion to the US economy.[5] In 1986, 924 liver transplants and 140 pancreatic transplants were performed in the United States. Even if the organ donor pool were to double or triple, it would fall far short of answering the need. Thus, major problems with organ transplantation as currently practiced include donor scarcity, expense, technical difficulty, and labor-intensive and complex care.

Surgeons have recognized these shortcomings for many years and have looked beyond transplantation for solutions. Paul Russell, MD (Fig 5, left), from our midst summarized new approaches to organ replacement when he wrote a review article on selective cell transplantation in 1985.[6] He clearly stated that if there was an effective way to transplant only those important functional cellular elements of an organ, there would be many conceptual advantages over organ transplantation. Researchers have tried for 15 years to solve the problem of islet cell transplantation, for example. Many others have worked on hepatocyte transplantation as a way to support patients with liver failure.[7] And so, cell transplantation has become a conceptual alternative, although now it is still highly experimental. In the 1970s, John Burke, MD (Fig 5, right), grappled with the problem of the massive burn wound. He realized that early excision and coverage was the cornerstone of suc-

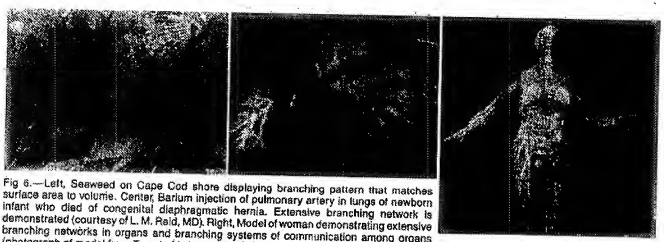
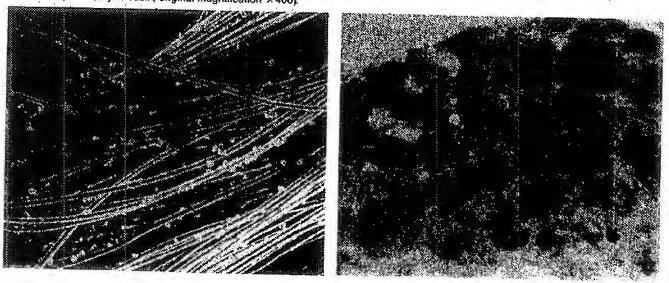


Fig 6.—Left, Seaweed on Cape Cod shore displaying branching pattern that matches surface area to volume. Center, Barium injection of pulmonary artery in lungs of newborn infant who died of congenital diaphragmatic hernia. Extensive branching network is demonstrated (courtesy of L. M. Reid, MD). Right, Model of woman demonstrating extensive branching networks in organs and branching systems of communication among organs (photograph of model from Toronto Museum of Science).

Fig 7.—Left, Scanning electron micrograph of polymer fibers with attached hepatocytes (original magnification ×121). Right, Implant of rat hepatocytes in omentum one week after implantation. Note active mitosis and vascularity (hematoxylin-eosin, original magnification ×400).



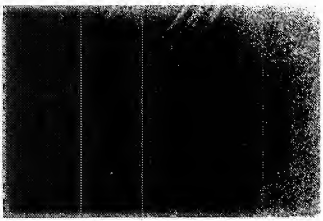


Fig 6.—Bovine aortic endothelial cells migrating into gel of complex biomatrix from polymer fibers (original magnification × 120).

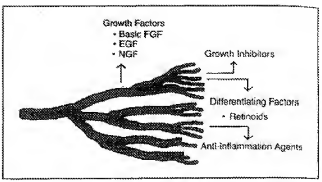


Fig 9.—Diagram demonstrating potential of slow release of biologically active macromolecules from polymer matrices. FGF indicates fibroblast growth factor; EGF, epidermal growth factor; and NGF, nerve growth factor.

cessful management. This approach, however, was limited by the availability of donor skin. Dr Burke began to conceptualize creating an artificial wound coverage, a matrix of neodermis that would be manufactured from natural elements. Working with Ionnas Yannas, MD, at Massachusetts Institute of Technology, Cambridge, Mass, an artificial dermis was designed from collagen and glycosaminoglycans. It was acellular and temporarily covered by a Silastic membrane. When placed onto an open burn wound, cellular migration and vascular ingrowth into the neodermis occurred, and the matrix was remodeled over time, becoming a living element. Epidermal elements from expanded skin grafts were gradually placed on the new dermis after vascular ingrowth occurred.[8] This concept and its successful use will clearly be one of the hallmark contributions to the management of patients in the latter half of the 20th century. Dr Burke and his colleagues were able to solve the problem of creating a new organ in two dimensions by using natural substances in a creative way.

We wondered whether we could apply these principles of cell transplantation and artificial matrix to three-dimensional systems in the design of visceral organs. What would be necessary? One would need dissociated parenchymal cells and an appropriate biodegradable scaffold that would allow the cells to remain viable by diffusion, promote vascular ingrowth, and permit cellular proliferation and function. The design of such a system is a major problem requiring expertise in cell and developmental biology, polymer technology, and biomedical engineering.

The rationale for such a proposal is based on several biologic observations. Every structure in living organisms undergoes constant renewal, remodeling, and replacement. Dissociated structural cells placed in cell culture tend toward reforming their structures. Their ability to do so depends on the conditions and cues provided in culture. Examples include endothelial cells that will form tubes in vitro and biliary cells that will form ducts in vitro.[9,10] Normal organ parenchymal cells are anchorage dependent. If dissociated cells are placed into mature tissue as a suspension without cell attachment, they may have a difficult time finding attachment sites that will allow for proper polarity and cell function. This may limit the total number of implanted cells that would remain viable to organize, proliferate, and function. Finally, but very important, tissue cannot be implanted in volumes of greater than 2 to 3 mm³ because nutrition, gas exchange, and elimination of waste products is limited by this maximum diffusion distance.[11] We evolved the concept of the engineered creation of a new organ in situ by placing functional, dissociated cells onto biodegradable artificial polymers in culture and then placing this polymer-cell scaffold into a host where vascularization, growth, and function could occur. We have termed this process *chimeric neomorphogenesis*.[12]

The technique involves the harvest of the appropriate parenchymal cell type and placement into cell culture on polymer matrices. The ability then exists to manipulate those cells while in culture. For example, one could expand the number of cells in culture, offering the opportunity of using a small sample of the patient's own cells and allowing proliferation to achieve adequate numbers to replace lost function. One could insert missing genes for protein products such as factor VIII using the methods of genetic engineering. One might also manipulate antigenic surface determinants or eliminate unwanted antigen-presenting cells, thereby decreasing the likelihood of immune rejection. The cell-polymer scaffolds are then placed back into patients in appropriate locations under the proper conditions. The major components of this technique are (1) the use of biodegradable polymers; (2) cell viability supported temporarily by diffusion; (3) proliferation and organization of cells; (4) vascularization of the growing cell mass; and (5) proper cell function in the context of new structure. One must emphasize the importance of vascularization to allow cells to have adequate nutrient, gas, and waste exchange. The design of the polymer scaffold must allow all cells access to the environment until vascular ingrowth occurs.

This work has been done in close collaboration with Robert Langer, SeD, and his group at the Massachusetts Institute of Technology. The initial design was that of a small wafer of biodegradable polyanhydride. Cells were seeded in a monolayer onto the wafer in culture and then placed into a recipient animal while on the disk. Our initial experiments were not very successful. We thought this was most likely due to cell number and cell density that were inadequate for successful engraftment. In this context, we began to address the question of growth of multicellular organisms. How does Mother Nature solve the problem of three-dimensional growth? As a mass of cells enlarges, the surface area increases only as the square of the radius, but the volume increases as the cube of the radius. How does nature tackle this mismatch so that the cells on the interior can be nourished? Nature uses branching networks to achieve this goal of matching surface area to volume. She

uses it both in the animal and the plant worlds. All organs are composed of intertwined branching networks and all communication between organs is accomplished by branching systems. Indeed, animals are structurally not much different from plants in this fundamental repeating pattern. To be successful in their niche, animals must think, react, and move; hence, the developed nervous system, the covering of the skin, and the mobility allowed by a musculoskeletal system (Fig 6). But, in essence, we are branching networks, much as plants are.[13]

Several experiments have been performed to test the feasibility of this concept. Biodegradable polymers of fiber networks are constructed. Cell suspensions from the liver, intestine, or pancreas are placed on polymers and the polymer-cell scaffold is maintained in culture for four days, and then it is reimplanted in the animal. Sites tested have included the omentum, retroperitoneum, and subcutaneous tissue. Figure 7, left, is a scanning electron micrograph of dissociated hepatocytes that have been placed onto polymer fibers of polyglactin and cultured for several days. There are many healthy hepatocytes and others that appear to be degenerating. Figure 7, right, is a hepatocyte implant in the omentum one week after engraftment. It shows very viable and healthy hepatocytes, as well as mitotic figures indicating cell proliferation in the growing mass. Visible as well are blood vessels throughout the implant and evidence of bile canaliculi as specilized areas of the hepatocyte membrane. We have placed aortic endothelial cells onto polymer fibers and suspended them in gels of complex biomatrix (Fig 8). We have observed proliferation of the cells on the matrix and migration of the cells off of the fibers into the gel in an organized manner reminiscent of capillary branching.

The polymers we have employed are man-made and therefore allow great flexibility in composition, configuration, and control. We can engineer tensile strength, rate of degradation, and suitability for cell attachment. We may be able to modify the inflammatory response and cell attachment by modifying the material or by coating it. Growth factors, growth inhibitors, and differentiation factors can be incorporated directly into the polymer matrix (Fig 9). As the polymer erodes, these factors can be released in a biologically active form to stimulate the tissue, signaling proliferation or differentiation. The polymers can also be placed with cells attached into three-dimensional gels of collagen or complex biomatrices; they then may be studied with time-lapse video photography. This should allow the in vitro study of cell-cell interactions. We are now rigorously studying optimal conditions for cell attachment. By preconditioning polymer fibers with various buffers, the polymer surface is roughened, thereby increasing cell attachment. We have also coated polymer fibers with collagen, gelatin, and fibronectin to increase cell attachment.

The year 2000 marks not only the beginning of a new century but the dawn of a new millenium. Cell transplantation holds great promise in the treatment of many diseases. Much work needs to be done, but our hope is that someday cellular chimeras will provide replacement tissue for patients as an alternative to organ transplantation as currently practiced.

References

1. Murray JE, Merrill JP, Harrison JH: Renal homotransplantation in identical twins. *Surg Forum* 1955;6:432-436.
2. *Transplantation Summary.* Washington, DC, Office of Health Technology Assessment & National Center for Health Services Research, US Public Health Service, May 1987.
3. Vacanti JP, Lillehei CW, Jenkins RL, et al: Liver transplantation in children: The Boston center experience in the first 30 months. *Transplant Proc* 1987;29:3261-3266.
4. *Fact Sheet, American Liver Foundation, 1986.* Cedar Grove, NJ, American Liver Foundation, 1986.
5. Boxheim R: Brother, can you spare $14 billion? *Diabetes Forecast,* May-June 1986, pp 33-35.
6. Russell PS: Selective transplantation. *Ann Surg* 1985;201:255-262.
7. Ebata H, Mito M: Intraspleric fetal rat hepatic tissue isotransplantation. *Transplantation* 1985;39:77-79.
8. Burke JF, Yannas IV, Quimby WC, et al: Successful use of a physiologically acceptable artificial skin in the treatment of an extensive burn injury. *Ann Surg* 1981;194:413-428.
9. Folkman J, Haudenschild C: Angiogenesis in vitro. *Nature* 1980;288:551-556.
10. Morse MA, Vacanti JP: Time-lapse photography: A novel tool to study normal morphogenesis and the diseased hepatobiliary system in children. *J Pediatr Surg* 1988;23:69-72.
11. Folkman J, Hochberg MM: Self-regulation of growth in three dimensions. *J Exp Med* 1973;138:745-753.
12. Vacanti JP, Morse MA, Saltzman WM, et al: Selective cell transplantation using bioabsorbable artificial polymers as matrices. *J Pediatr Surg* 1988;23:3-9.
13. MacDonald N: *Trees and Networks in Biological Models.* New York, John Wiley & Sons Inc, 1983.

Related Proceedings Appendix

None